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Source: Journal of Wildlife Diseases, 14(4) : 409-415

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-14.4.409

BONE MARROW AND HEMATOLOGIC VALUES OF WILD RACCOONS[1]

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Abstract: Blood and bone marrow samples were obtained from wild raccoons in the State of Connecticut to determine leukocyte and erythrocyte counts, hemoglobin, hematocrit, and peripheral blood and bone marrow differential counts. Calculations were made to determine mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.

INTRODUCTION

The raccoon, *Procyon lotor,* has adapted well to the civilized habitat of man and may serve as an indicator of certain environmental pollutants and several zoonotic diseases.[3,5,10,12]

Some information on normal blood values of wild raccoons has been published, but bone marrow cytologic studies and differential counts have not been made. Cytologic data have been published for captive raccoons in Canada,[11] and blood values have been determined for free-ranging raccoons in Florida.[9]

When used in conjunction with hematologic findings, bone marrow values are useful aids in the diagnosis and study of several diseases.[6,9] The purpose of this study is two-fold: (a) to supply additional information of the hematology of raccoons, and (b) to develop methods and guidelines for the study of bone marrow in wild raccoons. Knowledge of the hematopoietic system will provide baselines for experimental studies, aid in the diagnosis of naturally occurring diseases, and be useful in monitoring certain environmental pollutants affecting the raccoon.

MATERIALS AND METHODS

Ten adult raccoons were trapped in wire cages and submitted live to the Northeastern Research Center for Wildlife Diseases. Anesthesia with ketamine hydrochloride[3] was used to facilitate handling.[4] Blood was collected by cardiac puncture and each sample was divided into two aliquots. A 40-ml sample was allowed to clot and the serum was separated and stored at -70 C for future serologic studies. Five ml were collected into a 5 ml tube containing 5.5 mg EDTA (ethylenediamine tetraacetic acid)[4] and used for determinations of leukocyte count (WBC), erythrocyte count (RBC), hematocrit (HCT), hemoglobin (HGB), and cytology by blood smears. Following exsanguination, each animal was examined at necropsy and the tissues processed for histopathologic study. At the time of necropsy, bone marrow smears were

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[3] Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, New York 13201, USA.

[4] Labtubes, Courtland Laboratories, Los Angeles, California 90053, USA.

made from the femur using a soft camel-hair brush.

Bone marrow samples were not obtained from one raccoon and blood was not available from another animal which died under anesthesia.

Smears of blood and bone marrow were fixed in absolute methanol for 5 min and stained with Wright-Giemsa stain. Eight to 10 drops of standard Wright's stain was left on the slides for 2 min, then diluted with an equal amount of a 1:10 dilution of stock Giemsa in distilled water. This combined stain was allowed to react for an additional 5 min. Slides were rinsed with tap water, air dried, and coverslipped.[13]

A Coulter counter (Model F_N) [5] was used to obtain total leukocyte and erythrocyte counts. Hemoglobin readings were made on an Hb-meter[6] and hematocrits were measured by the microhematocrit method. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the measured parameters.

RESULTS

Bone Marrow

The classification used is that of the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs.[1,2] It is based primarily on nuclear characteristics and secondarily on cytoplasmic features. A cell between any two stages was classified as the more mature form (Lewis, pers. comm.)[7,8] Five hundred cells from randomly selected fields were counted, with every cell in a given field included in the differential count. Cells damaged during preparation, and which could not be classified according to nuclear or cytoplasmic characteristics, were listed as degenerate forms. Differential values for bone marrow cells are shown in Table 1.

Erythrocytic Series

Rubriblast: This is the most immature of red blood cell series, measuring between 11 and 14 μm in diameter, and constitutes about 0.6% of the cells counted. It is a round cell having a large, centrally located, purple-blue nucleus with coarse chromatin and 1 to 3 distinct nucleoli. The nucleus comprises the greater portion of the cell mass and the cytoplasm is homogeneous and deeply basophilic.

Prorubricyte: This cell is smaller than the rubriblast, measures 9 to 11 μm, and constitutes about 3% of the cells counted. The nucleus to cytoplasm ratio is the same as the rubriblast. The nuclear chromatin is slightly more condensed than the previous stage and only nucleolar remnants remain. The cytoplasm remains deeply basophilic.

Rubricyte: This cell averages 5 to 8 μm in diameter but may vary in size and constitutes approximately 9.5% of the differentiated cells. The nuclear chromatin is more clumped than in the precursor stages, and stains unevenly. There is decreased basophilia of the cytoplasm and the appearance of perinuclear orange staining areas indicates hemoglobin synthesis.

Metarubricyte: The cell measures about 4 to 6 μm and comprises 19% of the differentiated cells. The nucleus is pyknotic, the cytoplasm is without basophilia, and it stains orange-gray due to hemoglobin synthesis. In normal

[5] Coulter Diagnostics, Hialeah, Florida 33010, USA.

[6] American Optical Co., Buffalo, New York 14215, USA.

[7] Lewis, H. B. 1977. Personal communication. Department of Pathology and Toxicology, Smith Kline and French Laboratories, Philadelphia, Pennsylvania 19101, USA.

TABLE 1. Differential marrow counts in 9 raccoons (%).

Cell types	Range	Mean	S.D.
Erythrocytic series			
Rubriblast	0.2 - 1.0	0.62	0.35
Prorubricyte	1.8 - 4.2	2.93	0.66
Rubricyte	5.6 - 13.8	9.53	2.55
Metarubricyte	11.4 - 29.2	18.79	6.07
Granulocytic series			
Myeloblast	0.0 - 1.2	0.54	0.48
Progranulocyte	0.4 - 3.6	1.48	1.11
Neutrophilic myelocyte	1.8 - 6.3	3.39	1.33
Eosinophilic myelocyte	0.0 - 1.6	0.77	0.55
Neutrophilic metamyelocyte	4.8 - 11.0	6.81	2.05
Eosinophilic metamyelocyte	0.4 - 4.1	2.19	1.43
Neutrophilic band	6.8 - 23.0	13.57	4.82
Eosinophilic band	1.6 - 9.2	3.70	2.57
Segmented neutrophil	11.0 - 32.0	21.16	7.52
Segmented eosinophil	1.4 - 10.2	4.63	2.61
Lymphocytic series			
Lymphocyte	1.4 - 18.0	5.81	4.79
Plasma cell	0.4 - 1.8	0.97	0.53
Monocytic series			
Monocyte	0.0 - 1.2	0.48	0.35
RE cell	0.0 - 0.7	0.23	0.21
Degenerate Forms	0.2 - 5.4	2.66	1.91
TOTAL ERYTHROID ELEMENTS	19.8 - 43.6	31.36	8.70
TOTAL MYELOID ELEMENTS	46.2 - 67.8	58.51	8.75
M:E RATIO	1.1 - 3.1	1.93	0.67

healthy raccoons, these cells rarely are found in the circulation.

Granulocytic Series

Myeloblast: This cell measures 15 to 17 μm in diameter, is round to ovoid in shape, and comprises 0.5% of the total cells counted. It has a large, pale nucleus with a fine chromatin network and two or more distinct nucleoli. The myeloblast has more cytoplasm than the rubriblast and is only slightly basophilic.

Promyelocyte: This is a large cell measuring 11 to 14 μm and comprising about 1.5% of the cells. Nucleolar remnants are seen in the chromatin network. The promyelocyte contains more cytoplasm than the myeloblast and primary azurophilic granules are now present.

Myelocyte: The nucleus is eccentric and is bordered by a prominent pale-staining Golgi zone. The nuclear chromatin remains fairly immature but no nucleoli are present. The cytoplasm is pale bluish gray and specific granules (neutrophilic, eosinophilic, or basophilic) are now apparent. The neutrophilic granules are pale spherical structures which are not readily visible. The eosinophilic granules are prominent, uniformly round, bright red granules. No basophilic myelocytes were seen in this study. The neutrophilic myelocyte measures 11 to 13 μm, and amounts to 3.4% of the total. The eosinophilic myelocyte is the same size and accounts for 1.3% of the cells.

Metamyelocyte: This cell measures 7 to 12 μm. The nuclear chromatin stains

unevenly and tends to clump. The nucleus is indented and eccentrically located. The cytoplasm is more mature and stains bluish gray, but the granules remain the same as for the myelocyte. Neutrophilic metamyelocytes comprise 6.8% of the total cells, and eosinophilic metamyelocytes comprise 2.2%.

Band Myelocyte: This stage is characterized by a horseshoe-shaped nucleus. The chromatin is condensed and stains unevenly. The cytoplasmic granules are as previously described. The band cells measure 7 to 9 μm. Neutrophilic bands comprise 13.6% of the total cells counted and eosinophilic bands 3.7%.

Segmented (Polymorphonuclear) Myelocyte: This is the most mature stage of the granulocytic series. The nucleus is constricted into many segments. The number of segments ranges from 3 to 6, increasing as the cell ages. Segmented neutrophils constitute 21% and eosinophils 4.6% of the marrow cells.

Lymphocytic Series

Lymphocyte: Only the mature lymphocyte is observed in the marrow and peripheral blood. This cell ranges from 7 to 15 μm and constitutes 5.8% of the total marrow count. The small lymphocyte has a round purple nucleus and scanty dark blue cytoplasm. The large lymphocyte has a greater amount of lighter blue cytoplasm and a somewhat larger nucleus.

Plasma Cell: About 10% of the bone marrow is composed of plasma cells, although none are found in the peripheral circulation. The plasma cell is 7 to 10 μm in diameter and is oval or rectangular in shape. The eccentric nucleus has coarse chromatin which stains unevenly and is frequently clumped along the nuclear membrane.

Monocytic Series

Monocyte: The monocyte constitutes about 0.5% of the marrow, and measures 10 to 14 μm in diameter. It has a bi-lobed nucleus with a very fine chromatin network and abundant "foamy", blue-gray cytoplasm, often containing vacuoles.

Reticuloendothelial (RE) Cell: This cell is 7 to 9 μm in diameter and comprises 0.23% of the total. It is an irregularly shaped cell with a round purple nucleus. The distinguishing feature of the RE cell is the cytoplasm which often contains cell debris and pigments.

Megakaryocytic Series

Megakaryocyte: This is the largest cell and ranges from 30 to 75 μm and varies greatly in shape. It is multinucleated with 20 to 30 nuclei remaining close together after dividing. There may be abundant blue cytoplasm with evidence of thrombocyte formation, or only scanty blue cytoplasm surrounding the nuclei.

Reticulum Cells

Reticulum Cell: This large, irregularly shaped cell is widely accepted as the stem cell precursor of the erythrocytic, granulocytic, and megakaryocytic series.[8] In the raccoon, it has a large, purple, unevenly staining nucleus with 1 to 3 prominent, blue nucleoli, and a pale blue cytoplasm with an irregular border. The cell is seen infrequently and is not included in the differential count.

The myeloid:erythroid ratio (M:E ratio) is an expression of the total myeloid cells, including segmented cells divided by the total nucleated red blood cells found in the marrow. The values in this study ranged from 1.1:1.0 to 3.1:1.0 with a mean of 1.93:1.0.

Peripheral Blood

The total white blood cell count ranged from $5.9 \times 10^3/mm^3$ to $16.5 \times 10^3/mm^3$, with 66% segmented neutrophils, 0.9% eosinophils, 29% lymphocytes, and 3.6% monocytes.

Erythrocytes ranged from 4.5 to 6 μm in diameter and stained pinkish-orange with an area of central pallor. In some

smears there were areas of erythrocytes in rouleaux formation but it is not known whether this was artifactual. Total red blood cell counts ranged from 5.29 × 10^6/mm^3 to 8.78 × 10^6/mm^3, with a combined mean of 7.32 × 10^6/mm^3.

Hematocrits from the animals tested ranged from 27 to 51%, averaging 40%, while hemoglobin values averaged 13.1 gm/100 ml, ranging from 10.0 to 18.5 gm/100 ml. MCV ranged from 48 to 62 μm^3, averaging 55 μm^3, the MCH from 15 to 21 $\mu\mu$g, averaging 18.1 $\mu\mu$g, and the MCHC from 30 to 37%, averaging 33%.

Generally thrombocytes appeared singly or in small clumps measuring 0.5 to 2.0 μm. They stained pale gray and were evenly dispersed throughout the smears.

Peripheral blood values for the animals are shown in Table 2.

Pathology

The most consistent pathologic finding was intestinal parasitism, notably ascariasis and ancylostomiasis. Parasitic granulomas resulting from migrating ascarid larvae were frequently noted in the mesenteric lymph nodes and liver. Occasional granulomas were seen in the lungs and lamina propria of the gastrointestinal tract. Mild to moderate eosinophilic infiltration was present in the lamina propria of the small intestine of all animals examined. In two raccoons, encysted forms of *Trichinella spiralis* were found in the musculature of the tongue. *Dracunculus insignis* was found in the subcutaneous tissues of the legs in several raccoons, and the corresponding regional lymph nodes in these animals had marked RE cell hyperplasia, with numerous neutrophils and eosinophils in the medullary sinuses. The prescapular and axillary lymph nodes of one animal were similar to the nodes of the animals having subcutaneous nematodes, but no parasite was found. Sarcosporidiosis and intestinal coccidiosis were observed in several animals but their presence did not elicit any reaction from the host.

One animal had moderate suppurative balanoposthitis and mild urethritis, and cystitis. Several small abscesses were found in the lungs of another.

Two raccoons had disseminated lesions of the central nervous system characteristic of distemper virus encephalitis.

Liver lead concentrations were determined by Flameless Atomic Absorption Spectrophotometry.[7] Two raccoons, one male and one pregnant female, had significant lead concentrations — 10 and 15 ppm, respectively (measured on a wet weight basis).

DISCUSSION

The results of the bone marrow and blood differential counts are summarized in Tables 1 and 2, respectively. There is a significant sex difference in the total erythrocyte count with males having a mean erythrocyte count of 7.77 ± 1.03 × 10^6 and females 6.42 ± 1.06 × 10^6. Statistically significant sex differences were not observed for the other parameters in this study.

Eosinophilic granulocytes accounted for 12% of cells enumerated in the bone marrow differential. This value is high when compared to that of the dog (3.6%) and the cat (2.1%), and may be interpreted as a response to chronic parasitism.[13] Necropsy and histopathologic findings support this conclusion since all animals had a moderate parasite burden. Although the bone marrow was actively producing eosinophils, few were seen in the peripheral blood. This finding may be attributable to the trauma experienced by the animals during trapping and transport to the laboratory.

As previously mentioned, two raccoons had significant liver lead concentrations; however, no hematologic change was observed which could be related to plumbism.[7,14,15] The pregnant

TABLE 2. Peripheral blood values in 9 raccoons.

Parameters	Range	Mean	S.D.
RBC (x 10^6/mm^3)	5.29 - 8.78	7.32	1.18
WBC (x 10^3/mm^3)	5.9 - 16.5	10.9	4.3
HCT (%)	27 - 51	40	7.1
HGB (gm/100 ml)	10.0 - 18.5	13.1	2.4
MCV (μm^3)	48 - 62	54.7	4.4
MCH ($\mu\mu$g)	15 - 21	18.1	1.9
MCHC (%)	30 - 37	32.9	2.3
Neutrophils (%)	45 - 88	66.4	16.8
Lymphocytes (%)	11 - 51	29.1	15.5
Monocytes (%)	1 - 8	3.6	2.2
Eosinophils (%)	0 - 3	0.9	—

TABLE 3. Comparative peripheral blood values in raccoons.

		Present study	Hoff et al.[9]	Kennedy[11]
Parameters	Sex	Mean ± S.D.	Mean ± S.D.	mean ± S.D.
RBC (x 10^6/mm^3)	M	7.77 ± 1.03	—	11.1 ± 1.28
	F	6.42 ± 1.06	—	11.1 ± 1.38
WBC (x 10^3/mm^3)	M	10.1 ± 4.47	14.4 ± 0.77	14.3 ± 1.4
	F	12.5 ± 4.3	17.3 ± 1.23	16.1 ± 4.4
HCT (%)	M	42 ± 6.4	40 ± 0.81	—
	F	36 ± 7.8	39 ± 0.79	—
HGB (gm/100 ml)	M	13.7 ± 2.2	12.2 ± 0.25	11.5 ± 0.4
	F	11.7 ± 1.5	11.8 ± 0.28	10.4 ± 0.9
Neutrophils (%)	M	65 ± 15.8	72 ± 3.1	27.8 ± 6.3
	F	69 ± 22.1	72 ± 2.5	45.7 ± 8.2
Lymphocytes (%)	M	30 ± 14.6	23 ± 2.6	66.8 ± 6.9
	F	28 ± 20.7	24 ± 2.4	49.3 ± 8.1
Monocytes (%)	M	4.2 ± 2.4	1 ± 0.3	1.2 ± 0.8
	F	2.3 ± 1.5	2 ± 0.5	0.8 ± 1.0
Eosinophils (%)	M	1.2 ± 1.3	2 ± 0.8	4.1 ± 2.2
	F	0.3 ± —	2 ± 0.6	4.3 ± 1.9

raccoon had significantly lower RBC and HCT values which were interpreted as a reaction to the stress of pregnancy, possibly compounded by chronic subclinical lead intoxication.

The values obtained by Hoff *et al.*,[9] Kennedy,[11] and those of this study are summarized in Table 3. Total leukocyte counts were lower than those compiled by Hoff *et al.*[9] or Kennedy.[11] Blood differential counts were comparable to those obtained from free-ranging raccoons in Florida yet differed from those of captive raccoons in Canada.[9,11]

Acknowledgements

The authors wish to thank Dr. John Anderson of the Agricultural Experiment Station, New Haven, Connecticut, for his help in trapping the raccoons.

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Received for publication 22 April 1978